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DIAGNOSING BEE DISEASES IN THE APIARY

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FOREWORD

Bees, like all other living creatures, are subject to diseases, and their manner of living in crowded hives makes it almost inevitable that any contagious disorder will spread within the hive or to other colonies unless it is detected and the appropriate treatment given.

Other publications of the Department furnish information on the methods of treatment. This circular tells where to look and what to notice in the examination of colonies for possible or suspected disease.

More than one disease may be present in a colony, therefore the beekeeper should not discontinue the search on finding the symptoms of one disease. Especially is it important that American foulbrood be detected if it is present in the apiary.

If the nature of the disease is not apparent, samples of brood comb or the adult bees should be sent to the State apiary inspector or the Bee Culture Laboratory of the Bureau of Entomology and Plant Quarantine, National Agricultural Research Center, Beltsville, Md., as directed on page 34.

IMPORTANCE OF BEE DISEASES AND THEIR RECOGNITION

Bee diseases are found throughout the United States wherever bees are kept. These diseases cause large annual losses in bees, honey, and equipment and very materially add to the cost of honey

production. Unless bee diseases are recognized and controlled, individual colonies or even those of entire apiaries may be seriously weakened or destroyed.

It is important that beekeepers recognize bee diseases in their early stages so that they can apply proper methods of treatment, since practically all the diseases are more or less contagious and can spread from diseased to healthy colonies. Some of the diseases cause only slight losses, and can, to a certain extent, be disregarded. Others, however, are serious, and prompt treatment is required to prevent their spread. Consequently it is necessary that the beekeeper be able to recognize even the less serious diseases so as not to confuse them with the serious ones. The symptoms of sacbrood and European foulbrood, for instance, are often confused with those of American foulbrood. Furthermore, American foulbrood may be mistaken for European foulbrood, and if the usual treatment for the latter is applied, the disease not only will not be arrested but is likely to spread to healthy colonies.

In recent years new bee diseases have been discovered. One of these, parafoulbrood, a serious brood disease, at present appears to exist only in limited sections of the South. It is highly desirable to prevent the further spread of these newly discovered diseases and, consequently, beekeepers should learn to differentiate them from the other more widely distributed diseases.

There are also a number of abnormal conditions of bees that at times cause heavy losses and can easily be confused with some of the diseases. It has recently been found that nectar or pollen, or both, from certain plants may cause the poisoning of brood and adult bees. Then, too, the symptoms of poisoning or other abnormal conditions of bees such as chilling, starvation, or the presence of brood of infertile queens or laying workers, can easily be confused with the symptoms of some of the diseases.

No attempt has been made in this circular to describe methods of treatment, since these are available in other publications of the Department of Agriculture and in State bulletins and other beekeeping literature. Abnormal conditions of bees that are often difficult to distinguish from diseases are not discussed in detail, since it is planned to describe them in another publication.

BROOD DISEASES

WHAT TO OBSERVE WHEN LOOKING FOR BROOD DISEASES

To identify the brood diseases, any dead brood found in the cells should be examined carefully. The appearance of the combs may indicate which brood disease is present, but final diagnosis should always depend upon the symptoms shown by the dead brood. Dead brood in open cells can be seen clearly if a comb is held so inclined that the direct light of the sun falls on the lower side and bottom of the cells (fig. 1). If there is no dead brood in the open cells, any sunken, discolored, or punctured cappings should be removed and these cells examined for dead brood.

When dead brood is found, the following important points should be determined: (1) Age of the brood when death occurred, (2) po-

sition of the dead brood in the cells, (3) color of the dead brood, (4) consistency of the dead brood in different stages of decay, (5) odor coming from the combs, and (6) odor of dead larvae in different stages of decay.

A chart or guide for use in diagnosing diseases of the brood of bees is given in table 1 (p. 22).

It should always be kept in mind that more than one brood disease may be present in a colony. Of first importance at all times is

FIGURE 1.—Inspecting combs for brood diseases. A convenient way to hold the comb while looking for dead brood. The arrow indicates the direction of the sun's rays, which should fall on the lower side and bottom of the cells.

the early discovery of American foulbrood. When a less serious brood disease is found, it should be determined whether or not American foulbrood also is present.

AMERICAN FOULBROOD

CAUSE

American foulbrood is an infectious disease of the brood of bees caused by a bacterium known as *Bacillus larvae*. It is the most destructive of the brood diseases, is very infectious, and diseased colonies practically always die. This bacterium causes the death of larvae and pupae by its growth and multiplication within the stomach. It also causes a typical decay of the dead brood. *B. larvae* resists drying, the action of chemicals, both high and low temperatures, and the dehydrating action of honey; consequently American foulbrood cannot be treated successfully except by burning the infected combs and bees.

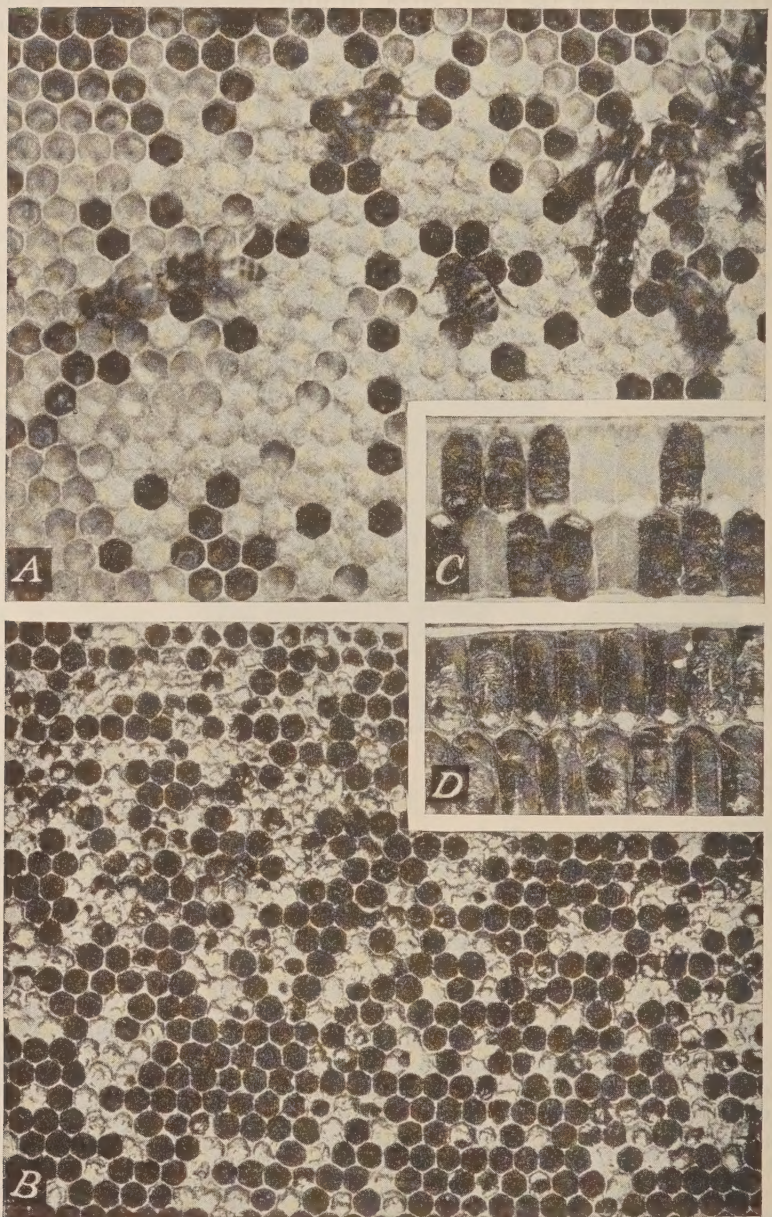

FIGURE 2.—American foulbrood: *A*, Healthy brood in new brood comb; *B*, old brood comb with an advanced case of American foulbrood; *C*, scales of larvae dead of American foulbrood on a cross section of a new comb; *D*, scales of larvae dead of American foulbrood in an old brood comb.

EFFECT UPON THE COLONY

The strength of a recently infected colony will not be noticeably affected, and there will be only one or a few dead larvae or pupae in sealed cells with slightly discolored or sunken cappings. The

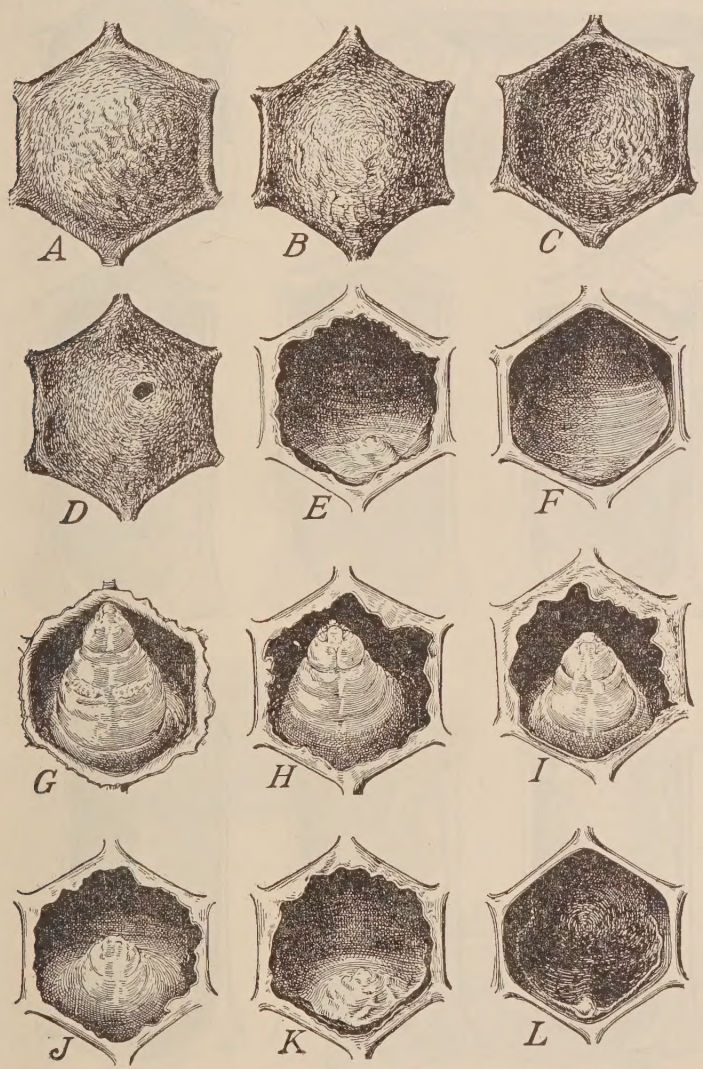

FIGURE 3.—Symptoms of American foulbrood: A, Normal capping over healthy larva; *B–F*, stages in the discoloration and removal of cappings; *G*, capping removed to show healthy larva; *H–L*, stages in the decay and drying of larvae killed by American foulbrood; oral views.

disease may not develop to a critical stage and seriously weaken the colony until the following year. At other times, however, the disease may advance more rapidly and seriously weaken or kill the colony the first season. If the disease has been present and active

for a considerable period, the colony will be noticeably weakened, and a large proportion of the cells (75 percent or more) will contain dead brood. *All weak, infected colonies found during any time of*

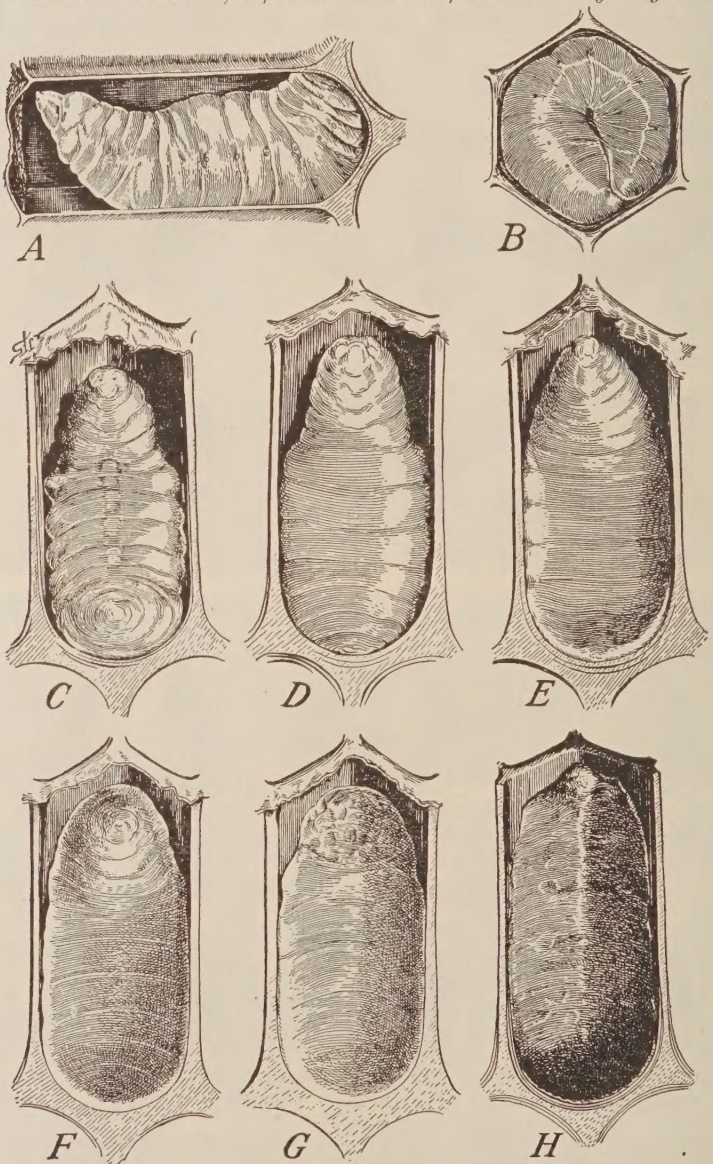

FIGURE 4.—Symptoms of American foulbrood in larvae: *A*, Healthy larva, lateral view; *B*, coiled larva recently dead; *C*, healthy larva, ventral view; *D–H*, stages in decay and drying of larvae, ventral views.

year should be burned at once to prevent spread of the disease through robbing. Under no circumstances should colonies be permitted to remain in the apiary until they have become seriously weakened by or die of American foulbrood.

APPEARANCE OF THE COMBS AND CAPPINGS

In healthy brood combs, where a normal queen has been laying, there is a certain regularity in the arrangement of areas containing eggs, larvae, pupae, and emerging bees (fig. 2, *A*), and the cappings are convex and uniform in appearance (fig. 3, *A*). In a colony in-

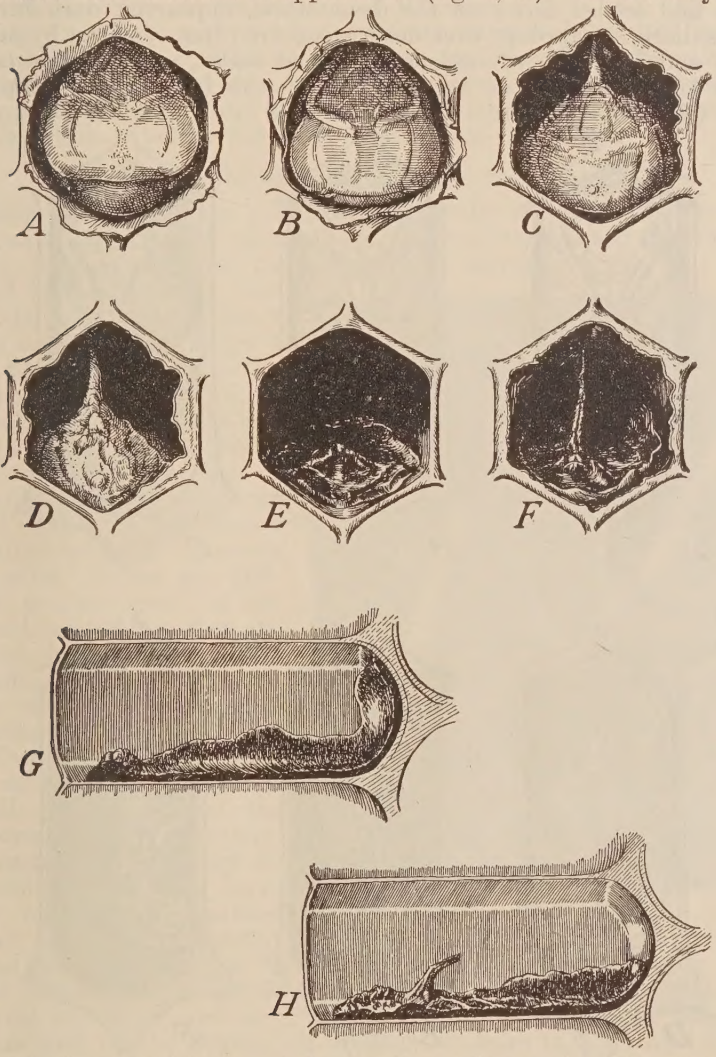

FIGURE 5.—Symptoms of American foulbrood in larva and pupae: *A*, healthy pupa; *B–F*, stages in the decay and drying of pupae; *G*, scale of dead larva, lateral view; *H*, scale of dead pupa, lateral view.

fected with American foulbrood the brood is more or less irregularly arranged, depending on the degree of infection. Great irregularity, due to the intermingling of cells of healthy brood with uncapped and capped cells of dead brood and cells with punctured and sunken cappings, is sometimes spoken of as the "pepperbox" appearance (fig.

2, *B*). Dead brood in cells with discolored, sunken, or punctured cappings (fig. 3, *B*, *C*, *D*) should always be studied carefully to determined whether death was caused by American foulbrood.

In advanced stages of the disease many of the cappings are punctured (fig. 2, *B*). Cappings may also be broken away at the edge and settled down on the dead brood, appearing dark brown and shining. Cappings over dead brood are often removed by adult bees, and in advanced cases many dried scales, as the remains of dead larvae and pupae are then called, can be seen in uncapped cells (fig. 3, *E* and *F*).

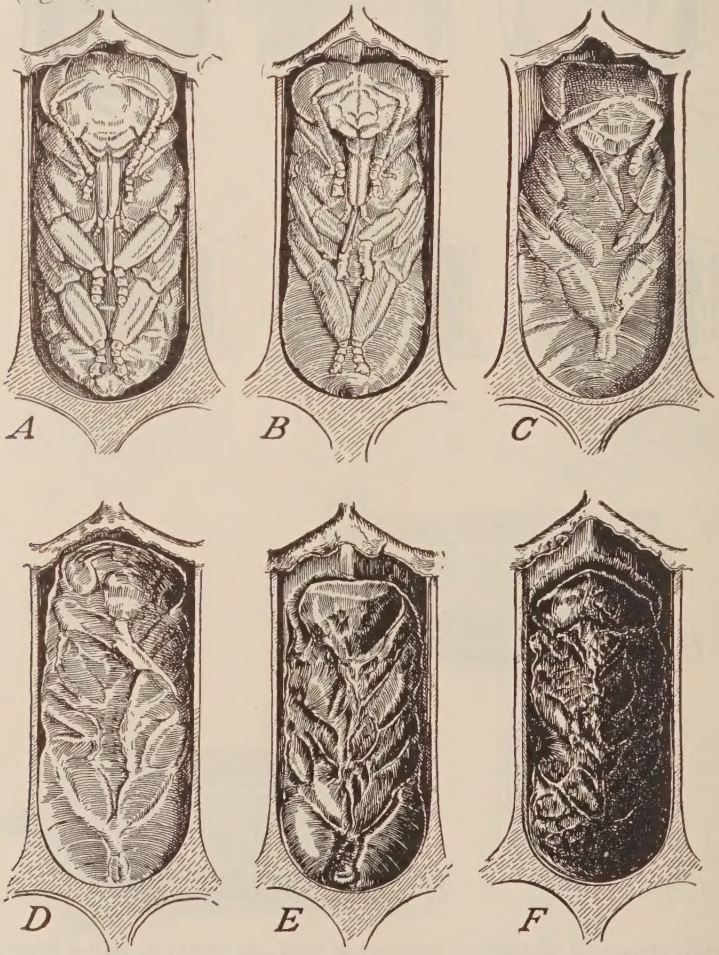

FIGURE 6.—Symptoms of American foulbrood in pupae: *A*, healthy pupa; *B–F*, stages in the decay and drying of pupae, ventral views.

SYMPTOMS SHOWN BY THE DEAD BROOD

KIND AND AGE OF AFFECTED BROOD

Usually only worker brood is affected, but occasionally drone and queen brood are also killed. Adult bees are never affected by this disease.

Death occurs quite uniformly after the larvae have been capped over, have spun their cocoons, and are fully extended on the floor of the cells, as shown by the healthy larvae in figures 3, *G*, and 4, *A*. Occasionally death occurs after the pupa has formed but before the body (except the eyes) is pigmented. (See healthy pupae in figs. 5, *A*, and 6, *A*.) In advanced cases a few larvae may die while coiled on the bottom of the cells (fig. 4, *B*), but only rarely does death occur when larvae are irregularly twisted on the side walls. Larvae killed by American foulbrood that are coiled or irregularly twisted often show symptoms similar to those of European foulbrood, and a laboratory examination may be necessary to determine whether the latter disease also is present.

COLOR AND CONSISTENCY OF THE DEAD BROOD

Soon after death the glistening white color of healthy larvae and pupae changes to dull white. About 2 weeks after death the color is very light brown, and the well-rounded appearance is lost. The dead brood gradually sink in the cells during decay and become darker (figs. 3 to 6), changing from a light coffee color to dark chocolate brown by the end of the fourth week. Scales are very dark brown or nearly black. The decay and drying of dead brood ordinarily require a month or more. Scales are difficult to distinguish in old brood comb, since they are about the same color as the comb (fig. 2, *D*); but in new comb they are readily distinguished (fig. 2, *C*).

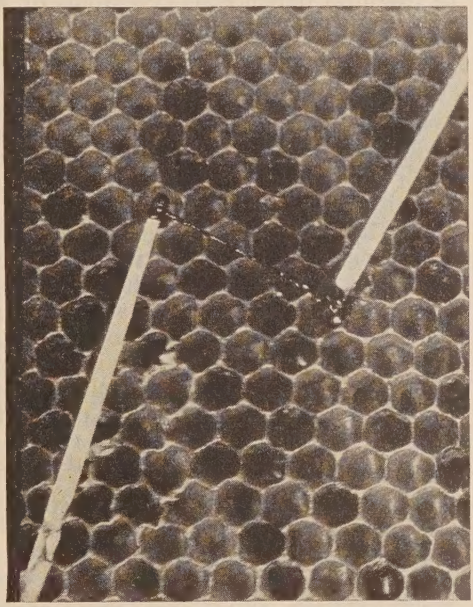

FIGURE 7.—American foulbrood; ropy remains of a decayed larva.

During the early stages of decay the body wall is easily ruptured, and the tissues are soft and watery. Occasionally the body divisions of the dead larva are more clearly marked than are those in healthy ones the consistency of dead brood becomes characteristically gluelike about 3 weeks after death. When a toothpick or match is thrust into a decayed larva and withdrawn, the decaying mass adheres and can be drawn out an inch or more in a glulike thread (fig. 7). Decayed larvae finally become dry and brittle.

APPEARANCE OF THE DEAD BROOD

The appearance and position in the cells of brood killed by American foulbrood are remarkably uniform. The dead larvae lie extended along the lower side wall with their posterior ends curved

part way up onto the bottom of the cells (fig. 5, *G*). There may be a small raised swelling near the head end of the scale, but this rarely is prominent. In advanced cases rows of cells contain dead larvae uniformly in this position.

When scales are numerous the disease can be diagnosed from their appearance alone. Scales can be seen extended along the lower side walls when the comb is held inclined so that a bright light falls on the lower side walls and bottoms of the cells (fig. 1). Occasionally cross markings which represent the segmentation of the larvae can be seen on the scales. When completely dried the scales are brittle and adhere so tightly to the cell walls that it is difficult to remove a scale without breaking it. When death occurs after pupation has started, the form of the pupa can be recognized in the scale (figs. 5, *H*, and 6, *F*). *The mouth parts of the dead pupa may protrude from the head of the scale and appear as a fine thread slanting slightly backwards into the cell and at times adhering to the upper wall* (fig. 5, *F*). The appearance of protruding or "stuck up" tongues is one of the most dependable symptoms of American foulbrood.

ODOR OF THE DEAD BROOD

In the first stages of decay, while the remains are still white, practically no odor is detectable. When the remains begin to turn brown and become ropy, however, an odor develops that is different from the typical gluepot odor characteristic of the advanced stages of this disease. In later stages, when the dead brood is brown and decidedly ropy, the familiar gluepot odor is always present, but it practically disappears when the scales are completely dry. In advanced cases, when much decaying brood is present, the gluepot odor can be detected even a foot or more from the combs. Since the odor of American foulbrood is characteristic, the use of the odor test is of considerable value in the diagnosis of doubtful cases. The odor can best be judged by holding some of the decayed remains on a toothpick at the entrance to the nostril and breathing deeply.

EUROPEAN FOULBROOD

CAUSE

European foulbrood is an infectious bacterial disease of the brood of honeybees. The bacteria grow within the stomach of infected worker, queen, and drone larvae and cause their death, but pupae are rarely attacked. Adult bees are not affected by this disease.

The earliest studies on European foulbrood seemed to indicate that it was caused by a rod-shaped bacterium, *Bacillus alvei*, which is commonly found in decayed brood. Later it was observed that lancet-shaped bacteria, different in shape and size from the rods and spores of *B. alvei* found in decayed brood, are usually present in large numbers in sick and recently dead larvae. This lancet-shaped bacterium, which was given the name *Bacillus pluton*, is now commonly considered to be the cause of European foulbrood. It has been found recently, however, that the rod-shaped *B. alvei* is capable of changing its form to a lancet-shaped bacterium resembling *B.*

pluton or other forms of bacteria found in larvae affected by European foulbrood. It seems probable, therefore, that *B. alvei* and *B. pluton* are only different forms of the same bacterium.

RACES OF BEES AFFECTED AND CONDITION OF COLONIES

Common black and Italian-black hybrid bees are more frequently affected by European foulbrood than are Italians, and weak colonies are usually more seriously affected than are strong ones. This disease frequently appears year after year in colonies of black or hybrid bees, and heavy losses may be suffered, but among Italian bees losses are usually unimportant. At times, however, European foulbrood spreads within strong colonies as well as within weak ones, and occasionally Italian bees are seriously affected.

EFFECT UPON THE COLONY

European foulbrood is most common in the spring, when brood rearing is at its height. Usually the earliest reared brood is not affected. Sometimes this disease appears suddenly and spreads rapidly within infected colonies. At other times it spreads slowly and does little damage. As a rule it subsides by midsummer, but occasionally it continues to be active during summer and fall, or it may reappear in the fall. A good honey flow seems to hasten recovery. In severe cases colonies are seriously weakened or killed. Usually the worker bees remove dead brood promptly (fig. 8, *M*); but in some colonies, particularly weak ones, it is allowed to accumulate.

SYMPTOMS

APPEARANCE OF THE COMBS

In mild cases and in early stages of European foulbrood the arrangement of the brood in the combs is not noticeably irregular. The degree of irregularity increases with severity of the disease and the length of time it has been present. In advanced cases open cells, which may be empty or contain eggs or healthy or affected brood, are irregularly scattered among cells of capped brood (fig. 9). Cells with discolored, sunken, or punctured cappings (fig. 8, *N* and *O*) may be present, but these are less common than in American foulbrood. Irregular arrangement of the brood is not a dependable symptom of European foulbrood, however, and final diagnosis should depend upon symptoms shown by the dead individuals.

APPEARANCE OF SICK LARVAE AND TIME OF DEATH

Sick larvae lose the plumpness and glistening white color of healthy larvae and become flat white. A faint yellow color, which is an important symptom, may also appear before death. Sick larvae may show abnormal movements and occupy an unnatural position in the cells.

The greater number of larvae die while coiled on the bottom of open cells (fig. 8, *A–I*). Many larvae also die at the age when they

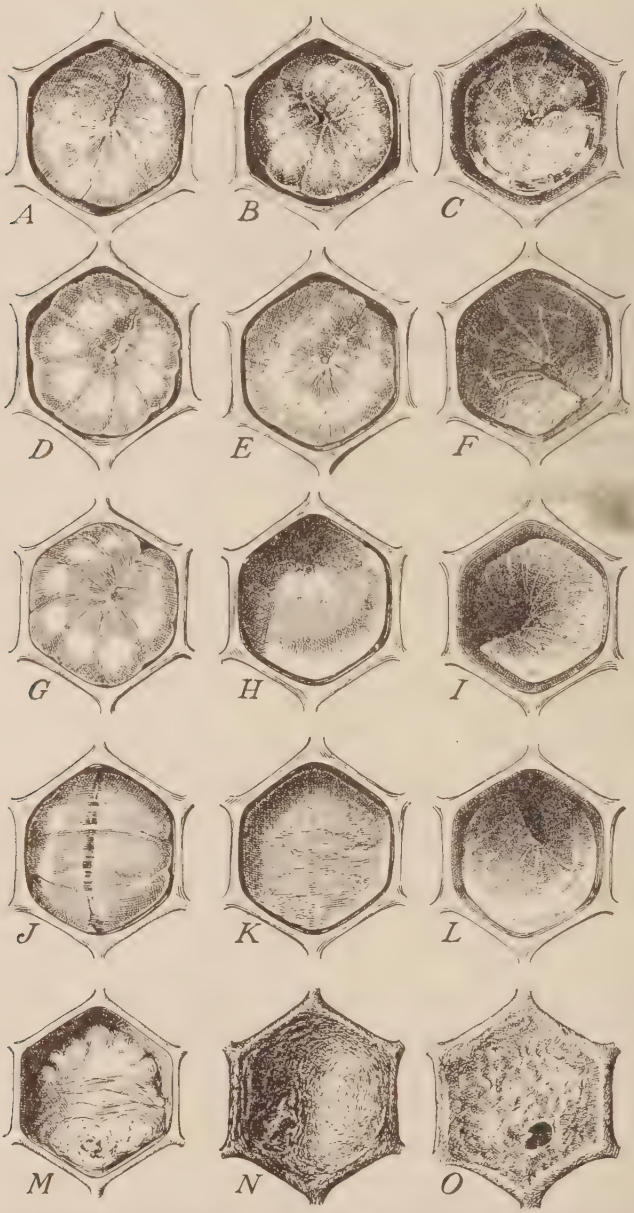

FIGURE 8.—Symptoms of European foulbrood. *A–C*, Larvae at the earliest age at which they may be attacked by the disease; *A*, earliest symptoms, *B*, more advanced symptoms, *C*, scale of a larva that died at this age. *D*, Healthy larva of slightly older age. *E*, Sick larva of this age. *F*, Scale of larva of this age. *G*, Healthy larva at the oldest age that larvae normally remain coiled on the bottom of the cells. *H*, *I*, Larvae of this age dead of European foulbrood. *J*, Healthy larva just before the cell is capped. *K*, *L*, Larvae of this age dead of European foulbrood. *M*, Dead larva that has been partly removed by the bees. *N*, Discolored and sunken capping over a dead larva. *O*, Punctured capping over a dead larva.

would normally be spinning their coccoons (figs. 8, *J–L*, and 10, *D–F*). Comparatively few larvae die while fully extended (fig. 10, *A*, *B*, *C*, *G*, *H*, *I*). Pupae are rarely affected by this disease.

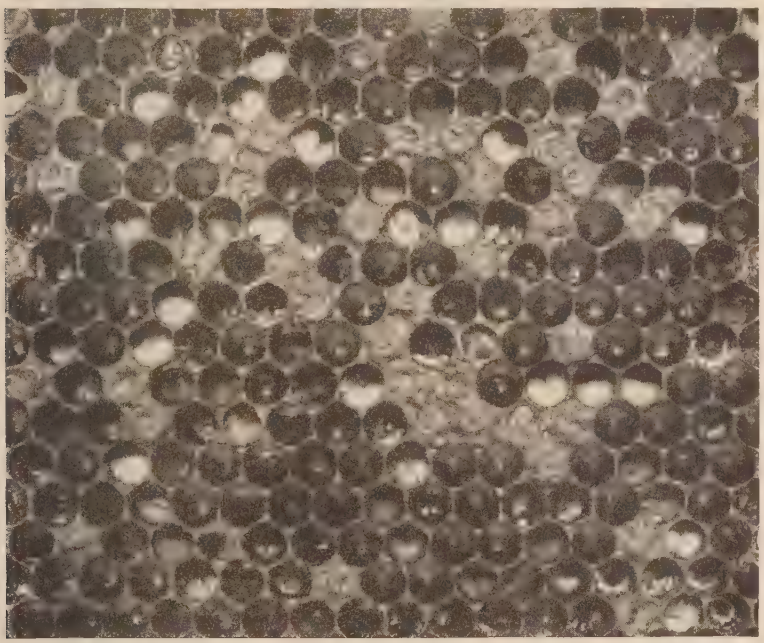

FIGURE 9.—European foulbrood; heavily infected comb showing larvae in various stages of disease and decay.

Larvae dead of European foulbrood, therefore, are usually coiled on the bottom of the cells but may be irregularly twisted or fully extended.

COLOR OF THE DEAD BROOD

Soon after death larvae become dull and grayish or yellowish-white. During decay the color deepens and may become brown or almost black. The tracheae, or breathing tubes, in dead larvae usually show more clearly than in healthy ones (fig. 8, *C* and *F*). They appear as radiating white lines in the dead coiled larvae and as narrow white lines across larvae that die while extended. A white line which crosses the radiating white lines can frequently be seen on the side of dead larvae. The prominence of the tracheae is a valuable symptom of European foulbrood but is not strictly dependable.

An elongated, dull grayish-white or yellowish-white mass can be seen through the skin along the back of sick and recently dead larvae. This mass is within the chyle stomach and consists of a turbid fluid that contains many bacteria. In healthy larvae, pollen in the stomach can often be seen through the skin along the back (fig. 8, *J*), but the color is usually of a brighter and deeper shade

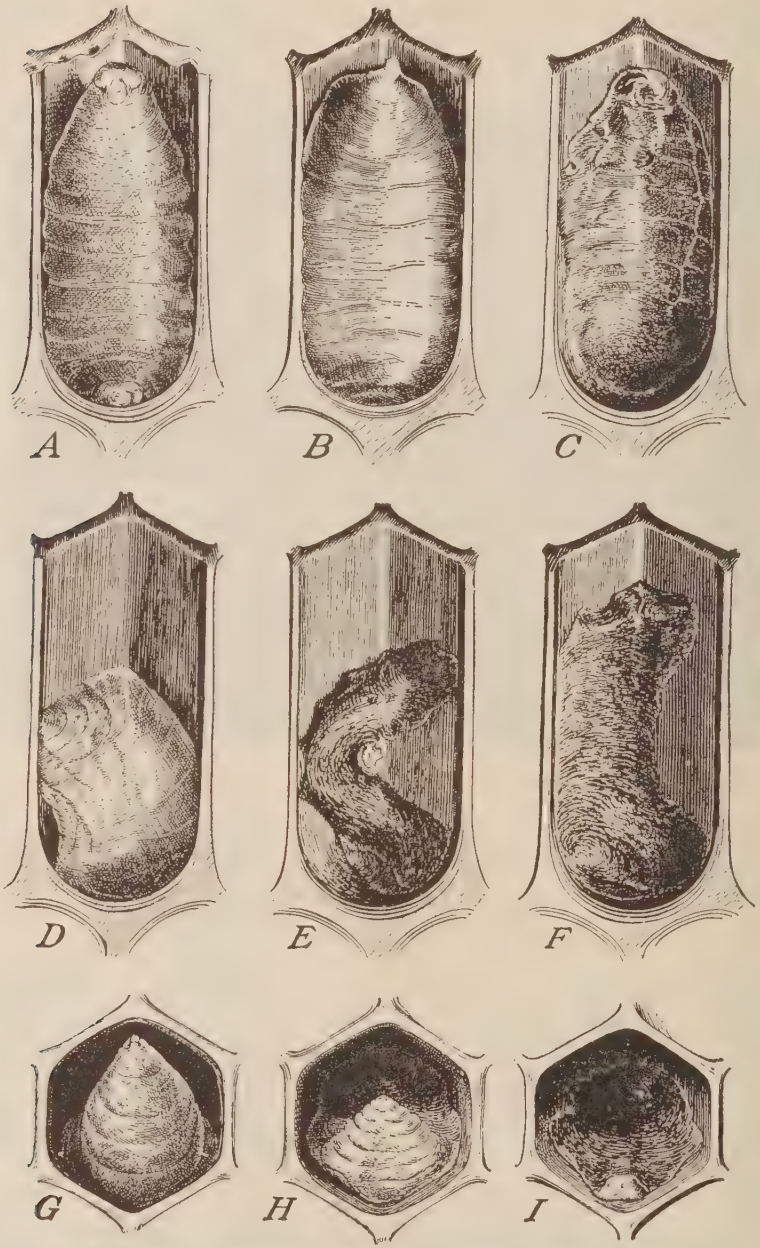

FIGURE 10.—Symptoms of European foulbrood: *A*, Ventral view of an extended larva recently dead of European foulbrood; *B*, extended larva partly decayed; *C*, scale of an extended larva; *D*, recently dead larva, and *E* and *F*, scales of dead larvae irregularly twisted; *G*, oral view of recently dead extended larva; *H*, partially decayed larva; *I*, scale of larva that died after straightening out.

of yellow than in affected larvae. Dissecting sick or recently dead larvae and examining the contents of the digestive tract helps in making a diagnosis after experience has been gained.

CHANGES CAUSED BY DECAY AND DRYING

The appearance of the dead larvae changes gradually during decay and drying. The gray and the yellow colors deepen during decay, but the depth of the color in scales varies considerably. Larvae that die before the cells are sealed dry rapidly, and decay is soon stopped; hence these scales are usually light colored. Larvae that die after the cells are sealed usually become dark brown or nearly black. Diagnosis of European foulbrood is more difficult after the dead brood is decayed and dry.

For a short time after death, larvae can be removed from the cells without tearing the skin. Within a few days the skin and other tissues become soft; and the larvae settle against the lower wall of the cells, and appear moist, melting, and flattened. At this stage in decay, larvae are somewhat translucent and watery and cannot be removed entire. Upon drying they become pasty, sometimes ropy, and finally rubbery or brittle. Scales of European foulbrood usually do not cling closely to the cell walls and are easy to remove.

Larvae that die of European foulbrood in sealed cells may become quite ropy and resemble larvae dead of American foulbrood. Since the bees remove dead brood from open cells first, it sometimes happens after disease ceases to be active that the brood which died in sealed cells is all that remains in the combs. When this happens it may be difficult to tell whether American foulbrood, or European foulbrood, or both of these diseases are present.

ODOR OF DEAD BROOD

The odors of European foulbrood cannot be accurately described but must be learned by smelling of the dead brood. When there are many decaying larvae in the combs an odor that is characteristic of this disease can sometimes be detected. Usually the odor of recently dead larvae is slight. A sour odor is sometimes present in partially decayed larvae. Some larvae, particularly those that die after they have straightened out and the cells are sealed, develop a putrid odor resembling the odor of decayed meat. This odor is nearly always present in larvae killed by European foulbrood which in other respects resemble larvae killed by American foulbrood. After the odors have been learned, the odor test helps considerably in distinguishing between European and American foulbrood when other symptoms overlap.

PARAFOULBROOD

CAUSE

Parafoulbrood is caused by bacteria which resemble the bacteria of European foulbrood. Worker, queen, and drone larvae and sometimes pupae are killed by the bacteria, which grow within the digestive tract, but adult bees are not affected by this disease. The spore stage of the bacterium found in affected brood is known as *Bacillus para-alvei.*

DISTRIBUTION AND RACES OF BEES AFFECTED

This disease has been found only in limited sections of North Carolina, South Carolina, Georgia, and Florida. All the races of bees common in North America are susceptible, but Italians appear to be more resistant than are common blacks and hybrids. Weak colonies are usually more seriously affected than strong ones, but heavy losses of brood may also occur in strong colonies.

EFFECT UPON COLONIES

Parafoulbrood progresses rapidly within some colonies and seriously weakens or kills them. In others it progresses slowly, the colonies are not noticeably weakened, and the disease disappears of its own accord. Some colonies clean out the dead brood promptly, while in others it is allowed to accumulate. In some apiaries only a few colonies will be diseased, while in others every colony will be

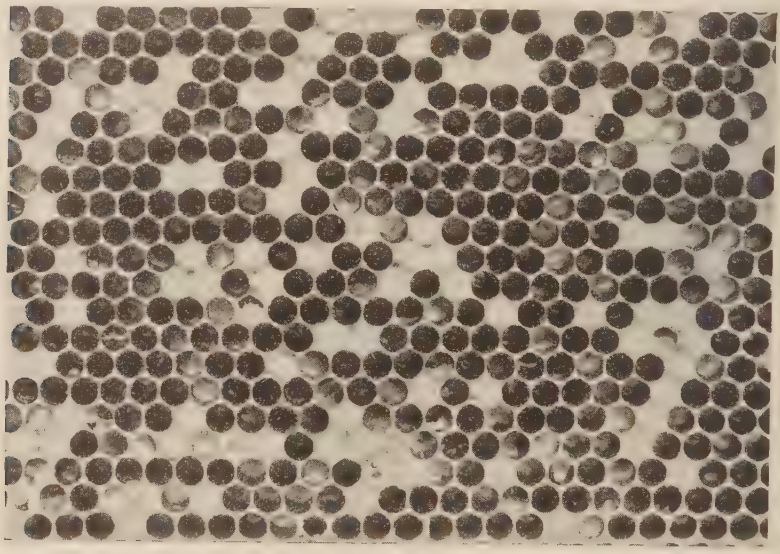

FIGURE 11.—Parafoulbrood: a heavily infected comb showing larvae in various stages of disease and decay after death.

affected. Loss caused by parafoulbrood may vary from the weakening of a few colonies to the loss of entire apiaries. This disease usually appears in the spring and disappears by midsummer, but occasionally colonies exhibit symptoms of the disease throughout the year, or there may be a slight increase of infection in the autumn. The first brood reared in the spring is not affected.

SYMPTOMS

APPEARANCE OF THE COMBS

Infected combs resemble combs with European foulbrood. The brood is more or less irregular, depending upon the amount of infection and the length of time the disease has been active (fig. 11).

Dead brood in open cells is removed by the bees sooner than that in sealed cells. Occasionally the bees increase the thickness of the cappings over dead brood in sealed cells. Such cappings appear dark, sunken, and greasy, and are sharply depressed in the center. Dead larvae may remain in these cells for months, or even over winter.

APPEARANCE OF SICK BROOD AND TIME OF DEATH

Sick larvae change from glistening white to dull or flat white, and a slight loss of plumpness may be noticed. They move uneasily in their cells and are often found in abnormal positions. A yellow discoloration occasionally appears before the larvae die.

Death from parafoulbrood usually occurs when the larvae are coiled or irregularly twisted in the cells, but many extended larvae and a few pupae are killed. The average age at the time of death is usually somewhat greater than in case of European foulbrood.

APPEARANCE OF DEAD BROOD

Larvae dead of parafoulbrood are coiled, irregularly twisted, or fully extended in the cells, depending largely on the age when death occurs. Usually the number of larvae and pupae that die in sealed cells is somewhat greater and the number of larvae that die while coiled is less than is the case in European foulbrood.

Larvae that die in open cells dry rapidly and usually form light-colored scales, although some become light brown, reddish brown, or dark brown. Larvae that die in sealed cells dry more slowly, and decay continues for a longer time. Many of these become reddish brown during decay and form dark-colored scales. In an occasional decayed larva or scale the tracheae show clearly. In sick or recently dead larvae the stomach can be seen through the skin along the back. The content of the stomach consists of a turbid grayish or yellow-gray fluid that contains many bacteria.

CONSISTENCY OF DEAD BROOD

Dead larvae soon become soft and watery. In capped cells some become decidedly ropy during decay and form dark reddish-brown or brown scales of a leathery consistency. In open cells the larvae usually become pasty and later form light-colored brittle scales. In some dead larvae ropiness develops rapidly, while in others it develops slowly or is entirely absent. Ropiness in parafoulbrood often resembles this symptom in American foulbrood. When this occurs a distinction can usually be made by noting the color and odor of the dead brood.

The scales can be removed easily from the cells.

ODOR OF DEAD BROOD

Only a slight odor can be detected in recently dead brood, and most larvae have but slight odor during decay. Many dead larvae in sealed cells and also some in open cells, however, develop an intense putrid odor similar to that of European foulbrood but fre-

quently much more intense. It can sometimes be detected as soon as a decayed larva is removed from the comb, and can also be detected in the dry scales.

A reliable symptom of this disease is a reddish-brown color and ropy consistency of decayed brood, particularly when accompanied by a pronounced putrid odor.

SACBROOD

Sacbrood is caused by a filterable virus, an organism so small that it will pass through a porcelain filter and cannot be seen under the most powerful microscope. Infection in the case of sacbrood takes place by way of the alimentary canal. Both worker and drone brood may be affected. It has not been definitely determined

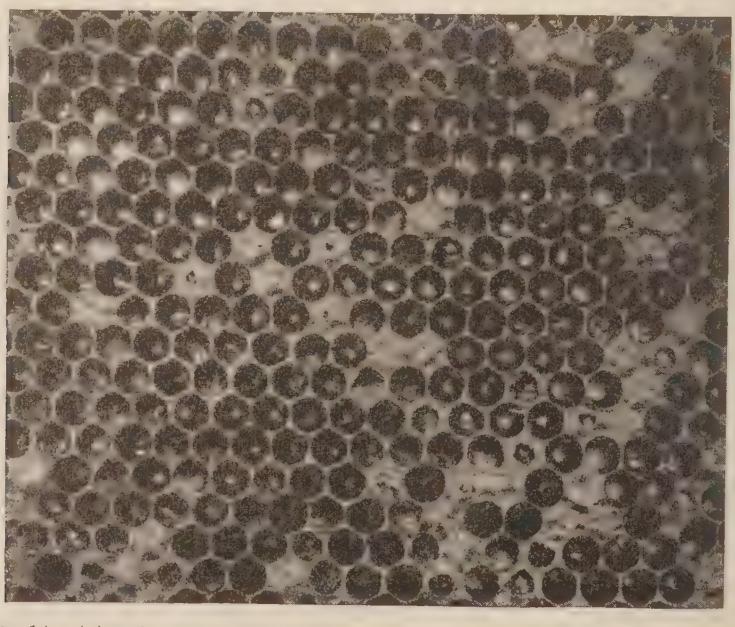

FIGURE 12.—A brood comb heavily infected with sacbrood, showing numerous dead larvae.

whether or not queen larvae are killed. Pupae are killed occasionally, but adult bees are not affected.

IMPORTANCE

Sacbrood is a widely distributed disease, but it usually does not cause serious losses. It is important, however, for beekeepers to recognize sacbrood so that it will not be confused with the foulbrood diseases.

Sacbrood may appear at any time during the brood-rearing season, but it is most common during the first half of the season, and practically always subsides after the main honey flow has started. In ordinary cases the colonies are not noticeably weakened by sacbrood, but in exceptional cases, when 50 percent or more of the brood is affected, they may be considerably weakened.

SYMPTOMS

APPEARANCE OF THE COMBS

In colonies with sacbrood the brood is slightly irregular. Scattered here and there among the healthy brood are cells containing dead brood (fig. 12). The cappings over dead brood are first punctured and later removed by the bees. The holes vary in size, and occasionally there is more than one. Sometimes the size and uniform shape of the hole indicate that the cell has never been completely capped. Dead larvae usually lie fully extended on the floor of the cell (fig. 13, *B–F*), showing the dark-brown heads through the openings (fig. 12). When these conditions are present the dead larvae should be studied carefully.

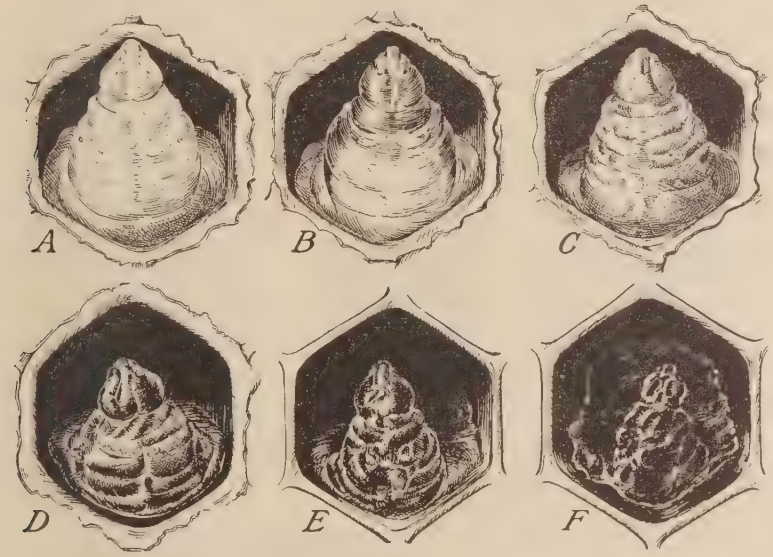

FIGURE 13.—Sacbrood: *A*, Oral view of healthy larva at the age when death usually occurs from sacbrood; *B–F*, stages in decay and drying of larvae dead of sacbrood.

AGE OF AFFECTED LARVAE

Death from sacbrood almost always occurs after the cell is capped and the larva has spun its cocoon and is motionless. At this stage the larva is fully extended on the floor of the cell. In heavily infected colonies a few coiled larvae may be killed.

COLOR AND ODOR OF THE DEAD BROOD

Shortly after death caused by sacbrood the color of the larva changes from the pearly white to a slightly yellowish color. This gradually becomes darker, beginning with the head and front third of the larva, which soon changes to a brown or grayish brown and later a dark brown. Scales are almost black for the entire length, the head end usually being darkest.

There is little, if any, distinctive odor associated with sacbrood, although watery, saclike larvae in the later stages may have a slightly sour odor.

CONSISTENCY OF DEAD BROOD

The skins of dead larvae remain tough, and are easily removed from the cells intact. The internal tissues at the same time become watery, but rarely show any indication of ropiness. Suspended in the waterlike liquid are numerous fine brown granules. When a dead larva is removed from the cell, liquid collects beneath the skin, which resembles a sac; hence the name sacbrood. As the larva dries, the skin becomes wrinkled, usually most noticeable in the front third (fig. 13, *C–F*). After thorough drying it forms a scale.

POSITION OF THE DEAD BROOD IN THE CELLS

Larvae killed by sacbrood almost invariably lie extended lengthwise with their backs on the floor of the cells (fig. 14, *G*). In contrast with American foulbrood (fig. 5, *G*), the head and front third of a larva dead of sacbrood is elevated while the tail end, as drying progresses, slumps partly down off the bottom of the cell. The raised head is a distinctive symptom of sacbrood. Since adult bees often remove recently dead larvae by biting off a piece at a time, occasional cells will be found in which only part of the dead larva remains.

THE SCALES

Scales of larvae dead from sacbrood can be removed from the cells with ease. They are dark grayish brown, or nearly black, and are hard and brittle with the head end turned sharply upward. The outline may be somewhat wavy. The back or lower surface is smooth and polished, while the upper surface is rough and somewhat concave. The lower surface takes the form of the cell walls and gives the entire scale a boatlike appearance often referred to as gondola-shaped or like a Chinese shoe.

INFECTION WITH TWO OR MORE BROOD DISEASES

In localities where two or more brood diseases are prevalent, more than one brood disease will occasionally be found in the same colony or even in the same comb. So far as is known a single larva is never affected by more than one disease. When American foulbrood is found in the same comb with European foulbrood or sacbrood, usually one of the diseases will be more prominent, at least in the active stages, which may cause the mixed infection to be overlooked, the beekeeper seeing only the most prominent symptoms. In cases where there is doubt or a suspicion that more than one disease may be present in the same colony, a laboratory diagnosis is desirable to prevent improper treatment. *Since American foulbrood is the most serious, a careful search for this disease should always be made even when another disease is known to be present.*

Table 1 gives in summary form the characters differentiating the principal brood diseases.

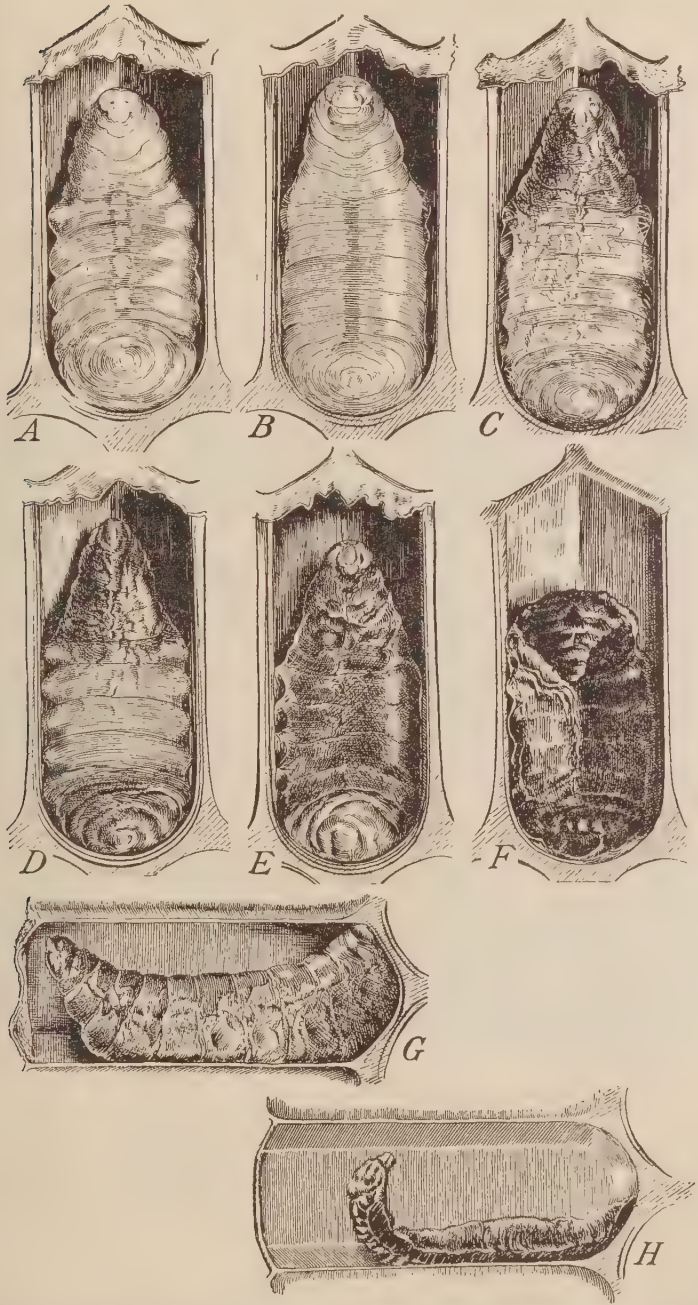

FIGURE 14.—Symptoms of sacbrood: *A*, Ventral view of a healthy larva at the age when death usually occurs from sacbrood; *B–F*, stages in the decay and drying of larvae dead of sacbrood, ventral views; *G*, lateral view of larva recently dead of sacbrood; *H*, lateral view of scale.

TABLE 1.—*Differentiating characters in the diagnosis of brood diseases*

Characters to observe	American foulbrood	European foulbrood	Parafoulbrood	Sacbrood
General appearance of brood combs.	Brood irregular; intermingling of capped, open, and punctured cells; much dead brood in capped cells, cells with punctured cappings, and cells uncapped by the bees.	Brood irregular; dead brood mostly in open cells.	Brood irregular; most of dead larvae in open cells; varying amount of dead brood in sealed cells.	Brood slightly irregular; dead brood mostly in cells with punctured cappings or in uncapped cells.
Appearance of cappings over dead brood.	Many punctured, sunken, and discolored.	Few cappings sunken, punctured, or discolored.	Cappings over dead brood punctured, discolored, sunken, or thickened and sharply depressed in the center.	Usually punctured.
Proportion of brood dead.	Varying from 1 or a few to 75 percent or more.	Varying from a few coiled larvae to most of the larvae in open cells, also a few larvae in capped cells.	Varying from few larvae to practically all the brood.	Small amount of brood dead; in severe cases, 50 percent or more.
Age at time of death.	Late larval and early pupal stage; rarely coiled stage.	Coiled stage; occasionally late larval stage.	Coiled larval stage; occasionally late larval and early pupal stage.	Late larval stage; occasionally coiled larval or pupal stage.
Position of dead brood.	Fully extended on floor of cell; tail turned up on bottom; head lying flat; great regularity.	Coiled on bottom or twisted on side walls; few larvae fully extended on floor of cell; very irregular.	Coiled on bottom, twisted on side walls, or fully extended on the floor of the cell; great irregularity.	Fully extended on floor; heads prominently raised; great regularity.
Color of dead brood.	At first dull white; then light brown; later coffee brown, dark brown, or almost black.	At first dull white, grayish white, or yellowish white; often becoming brown, dark brown, or nearly black.	At first dull white or grayish white, becoming light brown, brown, reddish brown, or dark brown.	Grayish to straw-colored, becoming brown, grayish black or black; head end usually darker.
Kind of brood affected.	Mostly worker; occasionally drone; rarely queen.	Worker, drone, and queen	Worker, drone, and queen	Usually only worker but sometimes drone.
Consistency of dead brood.	At first watery or slightly viscid, becoming ropy; finally brittle.	At first soft and watery; afterwards pasty, rarely viscid and ropy; scales tough, rubbery, or brittle.	At first soft and watery; in open cells becoming pasty and brittle; in capped cells frequently becoming ropy, finally tough or leathery.	Skin fairly tough; contents watery and granular; scales tough, brittle when completely dry.
Scales	Uniformly extended on lower side wall; tail curved up; dead pupae with tongue extended upward, often attached to upper cell wall; difficult to remove from cells.	Usually coiled on bottom of cells; often irregularly twisted; sometimes fully extended; tracheae often clearly visible; tough and rubbery; easily removed from cells.	Coiled on bottom, irregularly twisted on side walls, or fully extended in the cell; tracheae sometimes visible; easily removed from cells.	Uniformly extended on lower side wall; head prominently raised; outline wavy; grayish brown to nearly black; head darker; easily removed from cells.
Odor	No odor at first; distinctive odor different from gluepot odor in early stage of decay; gluepot odor in brown ropy remains and scales; odors constant.	Usually no specific odor in dead, coiled larvae; sour odor sometimes present in partly decayed remains; decayed-meat odor often present in decaying brood in sealed cells, finally penetrating and acidlike; odors variable.	Variable, resembling odor in European foulbrood but much more intense in ropy remains.	Absent or slightly sour.

FUNGOUS DISEASES OF BROOD

CAUSES

In addition to the diseases previously described, diseases of the brood of bees are caused by several different fungi. The most common of these are species of *Aspergillus*. In this country *A. flavus* attacks brood more frequently than other fungi. In Europe a fungus known as *Pericystis apis* causes a disease of brood known as "chalk brood." This fungus does not occur in North America.

IMPORTANCE

Normally only slight losses of brood are caused by fungous diseases. The small amount of brood that is killed is removed promptly by the worker bees and is rarely noticed by the beekeeper. Brood is most likely to become infected when moisture collects in the hive late in the winter and early in the spring, permitting fungi to grow over the combs.

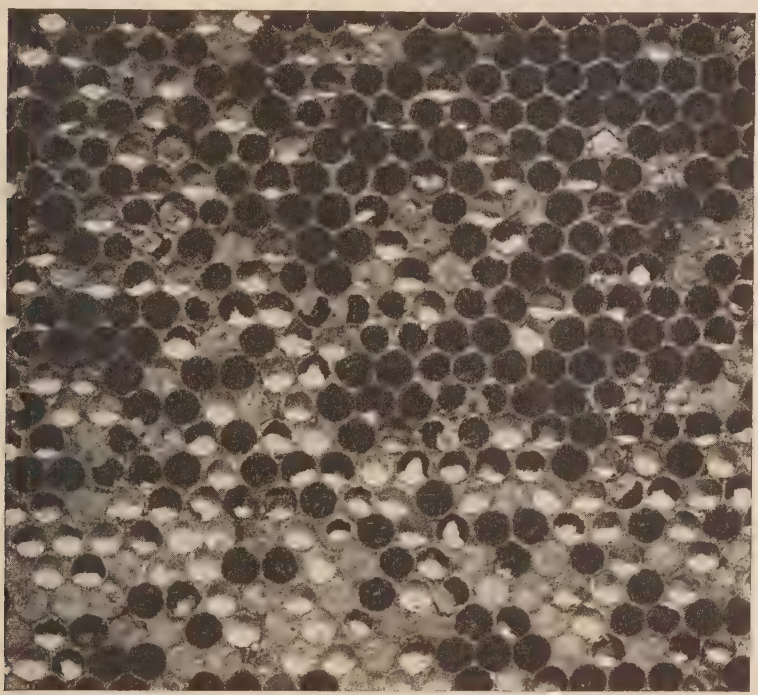

FIGURE 15.—Brood comb artificially inoculated with *Aspergillus flavus*, a fungus that kills the brood of bees. Dead larvae of different ages can be seen in the cells.

AGE OF BROOD AND RACES OF BEES AFFECTED

Brood of all ages, and also adult bees, are susceptible to fungous diseases. After the feeding period is passed, however, and the cells have been capped, brood is less likely to become infected. All the races of bees common in this country are susceptible.

APPEARANCE OF THE DEAD BROOD

A larva killed by a fungus becomes noticeably harder soon after it dies, and the glistening white changes to a dull creamy white. Later the dead larva becomes shrunken and wrinkled. The head end of a larva that dies after it has straightened out in the cell dries most rapidly and often curves upward at first but later tends to straighten out again (fig. 15). The fungus soon grows through the skin in a ring just back of the head and forms a sort of white collar. Within 1 or 2 days the fungus grows over the entire larva and forms a false skin which clings closely to the true skin. The color at this stage is chalky white. The fungus produces spores on the outer surface of the dead larva, and the white changes to a shade of green, black, or other color, corresponding to the color of the spores. Spores form earliest and most abundantly near the head end of

FIGURE 16.—Dead brood (mummies) killed by a pathogenic fungus.

dead larvae. The color of the spores deepens as they mature and fades as they become old and dry. After dead larvae and pupae have become dry they are known as mummies (fig. 16). In Europe the disease of bees caused by *Aspergillus flavus* is called "stone brood" on account of the hard texture of the dead brood.

DISEASES OF ADULT BEES

WHAT TO OBSERVE WHEN LOOKING FOR DISEASES OF ADULT BEES

No general rules can be given for the diagnosis of diseases of adult bees. Such diagnosis is made more difficult by the fact that at any time of the year many bees may die as a result of old age or abnormal conditions. Symptoms of the different diseases over-

lap, and usually a diagnosis cannot be made in the apiary. There are a few dependable symptoms of diseases of adult bees, however, which can be recognized without a microscope, and with good samples it is sometimes possible to make a diagnosis in the apiary.

NOSEMA DISEASE

CAUSE

Nosema disease is caused by a minute, single-celled animal parasite known as *Nosema apis*. Adult workers, drones, and queens are affected. Spores of *N. apis* enter the body of the adult bee with food or water. They germinate within the stomach and attack the tissues which line the stomach or mid-intestine, with varying harmful effects.

IMPORTANCE

Nosema disease is wide-spread and under conditions favorable for its spread causes extensive losses of adult bees. When accompanied by dysentery brought on by long winter confinement, the disease may spread rapidly within infected colonies and result in the death of the colonies late in the winter or in the spring; or heavy losses from Nosema disease may continue for weeks after the bees have been flying freely and dysentery has subsided. Infected bees usually perform their normal duties until they are too weak to continue. The shortened life of infected bees weakens or kills the colony.

SYMPTOMS SHOWN BY THE COLONY

The first noticeable symptoms shown by a colony heavily infected by *Nosema apis* are increasing restlessness of the bees and a weakening of the colony. When only a small number of bees are infected, the loss may be so gradual that it is not noticed. At other times the death rate among adult bees is very high, and the colony dwindles rapidly. The queen usually is among the last handful of bees to die. Nosema disease may appear annually at about the same time. During any time of year, however, colonies with bees infected by *N. apis* may be found that show no noticeable loss.

SYMPTOMS SHOWN BY INFECTED BEES

In the individual bee the symptom most commonly observed is inability to fly more than a few yards without alighting. Many bees will be seen crawling on the ground, on the bottom board, at the entrance, and on the top of frames when the cover is removed. Sometimes infected bees crawl actively long distances from the hive, or they may crawl up blades of grass in an effort to fly. At times they collect in small groups on the ground in front of the hive.

It is mostly the older workers that are killed, although drones, queens, and young workers may be attacked. At times the disease seems to be aggravated by periods of cold, damp weather, particularly in the spring when the bees cannot fly freely.

The legs of affected bees may be dragged along in crawling, as if paralyzed; and the rear wings may be unhooked from the front

wings and held at abnormal angles. Such bees are capable of only feeble fanning with the wings. The abdomen is often distended with feces and may appear shining or greasy.

APPEARANCE OF THE INTESTINAL TRACT

The intestinal tract of bees infected by *Nosema apis* is frequently swollen and discolored. When favorable specimens are at hand this symptom can be used for diagnosis in the apiary. If the bees are alive, or have just died, the entire intestinal tract can be removed as follows: Remove the head and hold the thorax with the thumb and forefinger, then grasp the tip of the abdomen with a pair of forceps and pull gently. By this procedure the entire intestinal tract can frequently be withdrawn from the abdomen.

In healthy bees the long, cylindrical mid-intestine is usually of a brownish-red, yellowish, or grayish-white color. Circular constric-

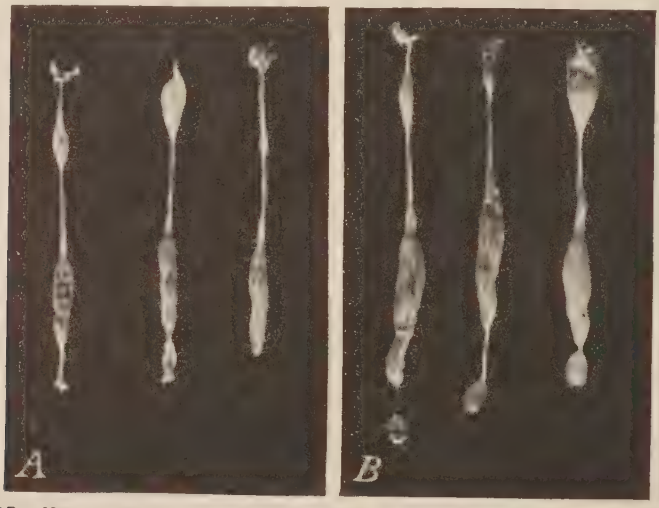

FIGURE 17.—Nosema disease: *A*, Intestines from healthy bees; *B*, Intestines from bees infected with Nosema disease.

tions show for nearly the entire length of the intestine (fig. 17, *A*), and the tissues are fairly tough and of a healthy appearance. When Nosema disease is present the mid-intestine swells (fig. 17, *B*) but finally shrinks to about normal size. Heavily infected intestines are usually of a dull grayish white, and some or all of the circular constrictions disappear (fig. 17, *B*). The tissues become soft and watery and are more easily crushed than are the tissues of healthy intestines. The fluid that flows from heavily infected intestines when they are crushed is whiter and more turbid than is the fluid from healthy intestines. After experience has been gained, it is often possible, when favorable specimens can be obtained, to make a diagnosis of Nosema disease in the apiary. There is considerable variation in the appearance of the mid-intestine of healthy as well as infected bees, however, and in many cases, particularly after the bees are dead, a microscopical examination is necessary for a diagnosis of Nosema disease.

ACARINE DISEASE

CAUSE

Acarine disease of adult honeybees is caused by a very small mite, *Acarapis woodi* Rennie. This mite lives as a parasite in the anterior thoracic tracheae (breathing organs), where it feeds directly upon the tissues of the bees. Bees are not noticeably injured by one or a few mites, but the mites breed and multiply within the trachea until they become very numerous. Heavily infested bees are unable to fly and soon die.

DISTRIBUTION

This disease of adult bees is not present in North America, but serious losses occur from it in Europe. Queens imported from Europe are sent directly upon arrival in the United States to the Government Bee Culture Laboratory at Washington. The attendant bees are examined for mites and other bee diseases. The imported queens are placed in new cages with young worker bees from the Government apiary before they are sent to the beekeeper who purchased them, in accordance with an act of Congress of 1922.[1] The rules and regulations and special rules incident to this act can be obtained by writing to the Bee Culture Laboratory, Bureau of Entomology and Plant Quarantine, National Agricultural Research Center, Beltsville, Md.

TRANSMISSION

The mites enter the tracheae at their openings (spiracles). When a few bees, or even one, of a colony become infested with fertile female mites, acarine disease may be transmitted to other bees within the colony. The mites mate within the tracheae, and later some of the females crawl out and enter the tracheae of other bees within the hive, thereby transmitting the disease. Acarine disease is thought to be transmitted from diseased to healthy colonies by the drifting of infested workers, or drones, or by robber bees. This disease may also be transmitted by requeening a colony with an infested queen.

SYMPTOMS

Infested bees are unable to breathe normally, and the walls of the tracheae and other tissues are injured. Bees that contain large numbers of mites are unable to fly and are known as crawlers. Crawlers usually leave the hive, when the weather is favorable, and

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[1] The act of Aug. 31, 1922 (Public, No. 293—67th Cong.), entitled "An Act To regulate foreign commerce in the importation into the United States of the adult honeybee (Apis mellifica)," provides as follows:

"* * * That, in order to prevent the introduction and spread of diseases dangerous to the adult honeybee, the importation into the United States of the honeybee (Apis mellifica) in its adult stage is hereby prohibited, and all adult honeybees offered for import into the United States shall be destroyed if not immediately exported: *Provided*, That such adult honeybees may be imported into the United States for experimental or scientific purposes by the United States Department of Agriculture: *And provided further*, That such adult honeybees may be imported into the United States from countries in which the Secretary of Agriculture shall determine that no diseases dangerous to adult honeybees exist, under rules and regulations prescribed by the Secretary of the Treasury and the Secretary of Agriculture.

"Sec. 2. That any person who shall violate any of the provisions of this Act shall be deemed guilty of a misdemeanor and shall, upon conviction thereof, be punished by a fine not exceeding $500 or by imprisonment not exceeding one year, or both such fine and imprisonment in the discretion of the court."

die outside. When large numbers of infested bees crawl from the hive at about the same time, the condition is known as mass crawling.

Bees often continue to work for weeks after they have become infested by mites, and acarine disease may be well advanced in a colony before symptoms are noticeable. The most commonly recognized symptoms are crawling and the loss of ability to fly. Crawling may come on gradually when the disease spreads slowly within the colony, or it may develop rapidly and result in mass crawling. After mass crawling has occurred, the colony is freed of most of the diseased bees and may appear to recover temporarily. Mass crawling often follows a period of unfavorable weather. Crawling is frequently accompanied by retention of feces, swollen abdomens, and unjointed wings.

FIGURE 18.—Acarine disease. Discolored trachea taken from the thorax of an infested bee. The mites that cause acarine disease can be seen through the tracheal wall. Magnified 75 times. (Photograph by J. Rennie.)

DIAGNOSIS IN THE APIARY

In healthy bees the tracheae are always pure white. In heavily infested bees the tracheae become bronzed or blackened in irregular spots. The presence of these spots is used as a symptom in diagnosis. With the aid of a lens that magnifies 6 or 8 times, the dark-colored spots can be distinguished. They may be few in number, or there may be so many that the trachea appears black (fig. 18).

In making examinations for acarine disease in the apiary it is best to use crawlers. The tracheae of bees killed by other disorders often

become black after a few days, while infested bees that are able to fly may not show the discolored spots on the tracheae. The head and front part of the thorax (prothorax) with the first pair of legs should be cut away and discarded. This will bring into view the first pair of breathing tubes, which are the ones most likely to be discolored if acarine disease is present.

SEPTICEMIA

CAUSE

Septicemia is a slightly infectious disease of adult honeybees. It is caused by growth in the blood of infected bees of a bacterium known as *Bacillus apisepticus*. This bacterium may be present in colonies, in the soil near infected colonies, or in water that has been in contact with bees killed by septicemia. Bees that become wet

FIGURE 19. Septicemia. Bees dead of septicemia dismembered by slight handling.

with soil water from about the hives may become infected. This is probably the most common way by which the disease spreads. The bacteria seem to enter the blood of bees by way of the breathing tubes. The presence of large numbers of bacteria in the food seems not to injure bees; but if a drop of water that contains the bacteria is spread over the entrance to the breathing organs (spiracles), disease and death from septicemia usually result. The disease does not spread readily from sick or dead bees to healthy ones unless plenty of moisture is present. The bacteria are soon killed by drying, and the disease rarely occurs under dry conditions. Several other species of bacteria and yeasts cause septicemia when they are placed in the blood of bees by puncturing the body covering, but they seem to be unable to gain entrance to the blood of uninjured bees.

SYMPTOMS

Bees die within a few hours after they have shown the first symptoms of septicemia. Sick bees leave the hive or are carried out by healthy workers. Sick bees resemble bees that are chilled, and their movements gradually become slower. Before death, the blood loses the normal clear, pale-brown color and becomes turbid and milky, owing to the presence of many bacteria. This symptom can sometimes be used in diagnosis in the apiary. By pulling off the head and abdomen of a dying or recently dead bee and pinching the thorax between the fingers a drop of blood oozes out that can be examined. Dead bees decay rapidly, the muscles of the thorax soon becoming soft and pasty, and the bodies have a characteristic putrid odor that is of some assistance in the diagnosis of this disease. Within 1 or 2 days the body, legs, wings, and antennae usually fall apart at the joints when the bees are handled (fig. 19).

EFFECT UPON THE COLONY

Only rarely are colonies noticeably weakened by septicemia, but many individual bees may be killed. Septicemia is less serious than Nosema disease or acarine disease.

AMOEBA DISEASE

CAUSE

Amoeba disease of bees, caused by a one-celled animal parasite, *Vahlkampfia* (*Malpighamoeba*) *mellifica*, was discovered a few years ago in Europe. This parasite grows in the excretory organs of adult bees. In 1927 it was found in two colonies of bees in the apiary of the Bee Culture Laboratory at Somerset, Md. In 1929 it was recognized in a sample of bees sent to this laboratory from California and in 1935 in a sample from Illinois. In Europe, amoeba disease was found only in colonies with Nosema disease, and it was suspected that the two diseases were in some way related. Nosema disease was not found, however, in one of the two colonies with amoeba disease in the apiary of the Bee Culture Laboratory, nor in the sample from California.

IMPORTANCE

Very little is known about the disease, but the infected bees are undoubtedly injured. Its economic importance is probably negligible, although this point has not been definitely determined.

SYMPTOMS

The disease cannot be recognized in the apiary by any symptoms. Dead bees that contain the parasites do not differ in appearance from bees dead of other causes. Field bees that appear entirely normal may also be infected. The parasites are found, often in large numbers, in the excretory organs (malpighian tubules) of the bees.

FUNGOUS DISEASES OF ADULT BEES

CAUSES

It has been known for many years that in Europe a disease of adult honeybees is caused by a common fungus, *Aspergillus flavus*. In North America it has recently been found that this same fungus and several others attack adult bees. *A. flavus* has already been noted as attacking brood (p. 23). When recently emerged bees are kept at a temperature about 12° or 14° below that of the brood nest they may be attacked and killed by *Mucor hiemalis*, a fungus closely related to the common black bread mold. Old bees are not affected by this fungus.

Spores of pathogenic fungi get into the digestive tract of bees with food or with water. If a bee comes in contact with fungus spores, some of them may cling to the mouth parts and be swallowed later. Nonpathogenic fungi are unable to grow within the stomach of bees, and the fungi themselves may be killed. Pathogenic fungi, on the other hand, grow readily. At first the fungus grows within the stomach, but later the muscles and other soft tissues are penetrated by numerous fungus branches, and death results. When dead bees are kept under moist conditions, the fungus may grow through the body wall and form spores on the outer surface (fig. 20).

FIGURE 20.—Adult workers and a drone bee killed by *Aspergillus flavus*. Spores of the fungus are seen on the bodies of the bees.

IMPORTANCE

Losses of adult bees caused by fungi are usually of little economic importance. When pathogenic fungi grow within the hive on combs, frames, dead bees, etc., late in the winter or early in spring, fungous diseases are most likely to cause significant losses. This can be

largely prevented, however, by providing good wintering conditions for the bees.

SYMPTOMS

The first noticeable symptoms are restlessness and weakness. Weakness increases until death occurs. A few sick bees may die in or near the hive, but they usually fly or crawl from the hive and seem intent upon getting as far away as possible before they die. For this reason mycosis of adult bees is likely to be overlooked, particularly when only a few bees at a time are affected.

By pressing the abdomen between the fingers, an increased firmness can sometimes be noticed at the time of death, but it is most noticeable a few hours later. It is unsafe to depend upon this symptom longer than about 1 or 2 days after death, since nonpathogenic fungi may produce similar symptoms in bees killed by other disorders.

"PARALYSIS"

CAUSE

The so-called paralysis of adult honeybees appears to be a slightly infectious disease that causes weakness, trembling, and death of the affected bees. The cause of paralysis has not been definitely determined, although various theories regarding the cause have been advanced from time to time. Recent work at the Government Bee Culture Laboratory indicates that paralysis is infectious.

IMPORTANCE

Paralysis of honeybees is a widely distributed disorder, but it causes greater losses in warm than in cold climates. Affected colonies usually recover after a short time, but in some cases the disorder continues throughout the season. In the Northern States it usually disappears or remains confined to one or a few colonies within an apiary, but in the South it sometimes spreads and causes considerable loss. The losses range from a few bees in mild cases to most of the bees of the affected colonies in malignant cases.

SYMPTOMS

Owing to the fact that the appearance of the sick and dead bees is not always the same, there seems to be a difference of opinion regarding the symptoms of paralysis. Other disturbances of adult bees may also have been mistaken for paralysis.

During the early stages of paralysis, affected bees remain on the combs and cannot be distinguished readily, except that the healthy bees often tug and pull at them excitedly. The sick bees make but little effort to defend themselves. Sometimes they offer food or attempt to escape by crawling away. Finally, they leave the hive and die outside or crawl into a corner of the hive or onto the top bars, where they remain until death occurs or until they are carried out of the hive by the healthy bees. Some affected bees die within a day or two after the symptoms have become noticeable, others linger for more than a week, while still others recover. The abdo-

mens of the sick bees are usually of normal size but often appear swollen or, less frequently, shrunken. Some of the sick bees retain their hairs until they die, whereas others become partially or entirely hairless, probably because their hairs are pulled out by healthy bees. Loss of hairs is accompanied by a darkening of the abdomen and thorax and a shiny or greasy appearance.

The most characteristic symptom of paralysis is weakness and a trembling or shaking movement of the body and wings, frequently accompanied by hairlessness and sprawled legs and wings. Sick bees that are motionless will sometimes show the trembling movements when disturbed. Some of the symptoms given here for paralysis are also present in other disorders of adult bees and cannot be depended upon alone for diagnosis. Trembling, weakness, and hairlessness, particularly when accompanied by dark, shining abdomens and sprawled legs and wings, seem to be the most dependable symptoms of paralysis. Bees in this condition tend to collect on top of the frames. A diagnosis can sometimes be made by carefully opening the hive, disturbing the colony as little as possible, and examining the bees on the top bars of the brood nest.

TRANSMISSION

It has been found by beekeepers that combs of brood from colonies with paralysis can be given to healthy colonies without spreading the disturbance and that the bees emerging from these combs remain healthy. In experiments at the Bee Culture Laboratory, combs of honey and pollen from affected colonies were placed in a healthy colony without paralysis being transmitted. When all the combs of an affected Italian colony were replaced with combs of brood from a healthy Caucasian colony, paralysis appeared among the young Caucasian bees within 2 weeks after the first of them emerged. Paralysis appeared to be transmitted when sick bees and young healthy ones were confined in the same cage. When young healthy bees were wet with water containing the macerated remains of affected bees, paralysis also appeared to be transmitted. The results of these experiments seem to indicate that paralysis is slightly infectious and spreads directly from sick or dead bees to healthy ones.

SENDING SAMPLES FOR LABORATORY EXAMINATION

If only a small amount of brood or a few bees are affected, or the symptoms are unusual, it is sometimes difficult to make a definite diagnosis in the apiary. Examination by laboratory methods is then necessary. It is also desirable at times to have diagnoses made in the apiary verified in the laboratory.

HOW TO PREPARE SAMPLES OF BROOD

In sending samples for laboratory examination, the following instructions should be followed: (1) Cut a sample of comb at least 4 by 5 inches in size. (2) Be sure that the sample contains as much of the dead or discolored brood as possible. (3) *No honey should be present*, and the comb should not be crushed. (4) Mail the

sample in a wooden (fig. 21) or strong cardboard box. *Do not use tin, glass, or waxed paper.*

Smears of dead brood and small crushed pieces of comb are frequently unsatisfactory for diagnosis but will be examined in case the foregoing instructions cannot be followed.

HOW TO SEND SAMPLES OF ADULT BEES

(1) Select, if possible, bees that are sick or recently dead; bees that have been dead for some time are not satisfactory for examination. (2) Send at least 50 bees in a sample; if poisoning by arsenicals is suspected, 200 or more bees will be needed for analysis. (3) Send bees in a wooden or strong cardboard box and *not in tin or glass.*

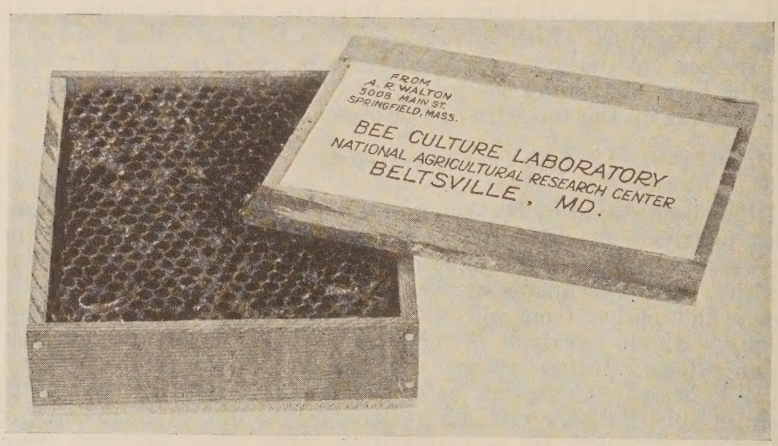

FIGURE 21.—How to send a sample of brood for laboratory examination.

HOW TO SEND SAMPLES OF TREATED COMB

(1) Send a sample not less than 4 by 5 inches in size if infection is heavy, or an entire brood comb if infection is slight. (2) Brood remains should be present in abundance. (3) Pack the comb in a clean wooden box as soon after treatment as possible. (4) *Do not send samples that contain honey.*

HOW TO ADDRESS SAMPLES

All samples should be addressed to the BEE CULTURE LABORATORY, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, NATIONAL AGRICULTURAL RESEARCH CENTER, BELTSVILLE, MD.

Your name and address should be plainly written on the box. If the sample is forwarded by an inspector, his name and address should also appear on the box.

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